Supporting Information

Properties of the modular model of a two-component signal transduction system

First, we will show that in Eq. (2) (see main text), $[\mathrm{R\text{ - }P}]$ (the concentration of phosphorylated regulator) approaches C_p in the limit of large $[\mathrm{R}]_{total}$ (total regulator concentration). Rewrite Eq. (2) as

$$2[\mathrm{R\text{ - }P}] = C_t + C_p + [\mathrm{R}]_{total} - \sqrt{(C_t + C_p + [\mathrm{R}]_{total})^2 - 4C_p[\mathrm{R}]_{total}} \qquad \text{(S1)}$$

(note that the quantities C_t and C_p are both positive).

Denote

$a = C_t + C_p$,

$b = 4C_p$,

$y = a + [\mathrm{R}]_{total}$.

With this notation, from Eq. (S1) we get

$$2[\mathrm{R\text{ - }P}] = a + [\mathrm{R}]_{total} - \sqrt{(a + [\mathrm{R}]_{total})^2 - b[\mathrm{R}]_{total}} = y - \sqrt{y^2 - b(y-a)}$$

$$= y - \sqrt{y^2 - by + ab}\,.$$

It follows that

$$\lim_{[\mathrm{R}]_{total}\to\infty} 2[\mathrm{R\text{ - }P}] = \lim_{y\to\infty} \frac{1-\sqrt{1-b/y+ab/y^2}}{1/y} = \lim_{x\to 0} \frac{1-\sqrt{1-bx+abx^2}}{x}\,, \qquad \text{(S2)}$$

where $x = 1/y$. Because both the numerator and the denominator in Eq. (S2) approach 0 as $x \to 0$, we use L'Hôspital's rule to calculate that limit. After differentiating both the numerator and the denominator in Eq. (S2), we get

$$\lim_{[\mathrm{R}]_{total} \to \infty} 2[\mathrm{R\text{ - }P}] = \lim_{x \to 0} \frac{b - 2abx}{2\sqrt{1 - bx + abx^2}} = b/2 = 2C_p \,. \quad \text{(S3)}$$

This expression proves that $[\mathrm{R\text{ - }P}] \to C_p$ in the limit of large $[\mathrm{R}]_{total}$.

Next, we will show that the variable $[\mathrm{R\text{ - }P}]$ in Eq. (2) (main text) is a monotonically increasing function of $[\mathrm{R}]_{total}$. Write Eq. (2) as $[\mathrm{R\text{ - }P}] = g([\mathrm{R}]_{total})$. We have that

$$g([\mathrm{R\text{ - }P}]) = a + [\mathrm{R}]_{total} - \sqrt{(a + [\mathrm{R}]_{total})^2 - 4C_p[\mathrm{R}]_{total}} \,;$$

$$g'([\mathrm{R\text{ - }P}]) = 1 - \frac{a + [\mathrm{R}]_{total} - 2C_p}{\sqrt{(a + [\mathrm{R}]_{total})^2 - 4C_p[\mathrm{R}]_{total}}} \,.$$

If $a + [\mathrm{R}]_{total} \le 2C_p$, then $g'([\mathrm{R\text{ - }P}]) \ge 1$, and, therefore, g is monotonically increasing. Let us assume that $a + [\mathrm{R}]_{total} > 2C_p$. In this case, $g'([\mathrm{R\text{ - }P}]) > 0$ will follow if

$$a + [\mathrm{R}]_{total} - 2C_p < \sqrt{(a + [\mathrm{R}]_{total})^2 - 4C_p[\mathrm{R}]_{total}}$$

or, equivalently,

$$(a + [\mathrm{R}]_{total} - 2C_p)^2 < (a + [\mathrm{R}]_{total})^2 - 4C_p[\mathrm{R}]_{total} \,.$$

After some algebra, this inequality is reduced to

$$-aC_p + C_p^2 < 0 \,.$$

Because $a = C_p + C_k > C_p$, the inequality above holds, proving that $[\mathrm{R - P}]$ in Eq. (2) is a monotonically increasing function of $[\mathrm{R}]_{total}$.

Now, we will prove that $[\mathrm{R}]_{total}$ is an increasing function of B in Eqs. (1)–(2). Let the pair $\{[\mathrm{R}]_{total} = R_0,\ [\mathrm{R - P}] = P_0\}$ be the solution to Eqs. (1)–(2) for a certain value $B = B_0$. Now, let $B_1 > B_0$, and let $\{R_1,\ P_1\}$ be the solution to Eqs. (1)–(2) for B_1. We need to prove that $R_1 > R_0$. Consider the functions $[\mathrm{R}]_{total} = f_B([\mathrm{R - P}])$ and $[\mathrm{R - P}] = g([\mathrm{R}]_{total})$ defined by Eq. (1) and Eq. (2), respectively. Because g is monotonically increasing, g^{-1} exists (and also is monotonically increasing), and Eq. (2) is equivalent to $[\mathrm{R}]_{total} = g^{-1}([\mathrm{R - P}])$. We have that $g^{-1}(P_1) = f_{B_1}(P_1) = R_1$. Further, $f_{B_0}(P_1) < f_{B_1}(P_1) = g^{-1}(P_1)$, which follows from the definition of the function f_B. At the same time, $g^{-1}(0) = 0 < f_{B_0}(0) = \tau B_0$. Therefore, due to the continuity of g^{-1} and f_{B_0}, these functions attain the same value X for some $[\mathrm{R - P}]_X \in (0, P_1)$. Therefore, the pair $\{X,\ [\mathrm{R - P}]_X\}$ is the solution to Eqs. (1)–(2) for $B = B_0$. Because this solution is unique (see Supplementary Information in Ref. 1), $X = R_0$ and $[\mathrm{R - P}]_X = P_0$. This implies that $P_0 < P_1$. Because g^{-1} is a monotonically increasing function, $R_0 = g^{-1}(P_0) < g^{-1}(P_1) = R_1$, Q.E.D.

Parameter values for the models

For the modular model of two-component signal transduction (Eqs. (1)–(2)), we used the following parameter values (taken from Ref. 1): $D = 40$, $C_t = 1$, and C_p, τA, and τB as indicated in the main text. For the simple model of positive autoregulation (Eqs. (3)–(4)) we used the following parameter values (taken from Ref. 2): $k_{-a} = 20 \ \text{min}^{-1}$, $H = 2$, $K = 5 \ \mu M^{-2}$, $k_d = 0.08 \ \text{min}^{-1}$, and k_1, k_2, k_a as indicated in the main text.

References

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Table S1. Bacterial strains used in this study.

Strain	Description	Source
EG13918	*phoP*-HA	Ref. 3
EG14338	Deletion of PhoP box in EG13918	Ref. 4
EG14943	Replacement of the PhoP box with -35 hexamer in EG13918	Ref. 5

Table S2. Primers used for RT-PCR.

Primer No.	Target genes	Sequences (5' - 3')
4443	*mgtA* forward	TAATTGCCACAAAACTTATG
4446	*mgtA* reverse	TCGCGGGAGAGGGGTGGGTT
4485	*mgtC* forward	GCGGGATTACGCACTAATGC
4486	*mgtC* reverse	GTCATGGAGCTCAGAATAAAAACG
6964	*pagC* forward	AAAAGATTAAATCGGAGCGGGA
6965	*pagC* reverse	TGACGCTCCATCCGCAATA
7013	*pagD* forwrad	ACATCATGCTTTTATGCTTTGGTC
7017	*pagD* reverse	AAACCAGAACAATGGCCTGAA
4489	*phoP* forward	GATGAAGACGGCCTTTCCTTAA
4490	*phoP* reverse	GAACCGGCAGTGAAACATCA
7108	*ugtL* forward	CGATTAGCTGACGGCTTTGTTT
7114	*ugtL* reverse	GATTTCTTCATTTTGAGCCTCCTC
7116	*yobG* forward	TGAAAAAATTTCGATGGGTCG
7118	*yobG* reverse	TGATATTAAACACCTGCGCCC
3023	*rrs* forward	CCAGCAGCCGCGGTAAT
3024	*rrs* reverse	TTTACGCCCAGTAATTCCGATT